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Anemophilous fungi isolated from libraries of educational institutions in the Northeast of Brazil

Fungos anemófilos isolados de bibliotecas de instituições de ensino da Região Nordeste do Brasil

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ABSTRACT

INTRODUCTION: Studies on airborne mycobiota from libraries show a wide variety of fungi, including those potentially pathogenic, offering occupational risk and allergic manifestations to their visitors. OBJECTIVE: To evaluate the occurrence of anemophilous fungi in libraries of educational institutions of basic and higher education in the city of Maceió, Alagoas State, Brazil. MATERIALS AND METHODS: Samples of the air were obtained from the exposure of 55 Petri dishes containing Sabouraud agar with chloramphenicol in three libraries of three educational institutions. The resulting fungal colonies were subjected to identification through the association of macroscopic and microscopic aspects, using microculture. Complementary phenotypic assays were also performed. RESULTS: A total of 351 colony-forming units (CFU) were obtained from the 55 analyzed samples, of which 331 (94.3%) were filamentous fungi and 20 (5.7%) were yeasts. The most frequent filamentous fungus species were *Penicillium* sp., *Cladosporium* sp., *Alternaria* sp., *Aspergillus* sp., and *Curvularia* sp., with a greater predominance of *Penicillium* sp. in a library that was not acclimatized, with 80 (22.7%) CFU. CONCLUSION: The results of this study show a great diversity of fungi with pathogenic and toxigenic potential, which can trigger allergic processes, thus confirming the importance of establishing hygiene and disinfection protocols in this type of environment.

Keywords: Fungi; Microbial Colony Count; Libraries.

RESUMO

INTRODUÇÃO: Estudos sobre micobiota anemófila de bibliotecas evidenciam ampla variedade de fungos, incluindo aqueles potencialmente patogênicos, oferecendo risco ocupacional e manifestações alérgicas a seus frequentadores. OBJETIVO: Avaliar a ocorrência de fungos anemófilos em bibliotecas de instituições de ensino da educação básica e superior da cidade de Maceió, estado de Alagoas, Brasil. MATERIAIS E MÉTODOS: Amostras do ambiente foram obtidas a partir da exposição de 55 placas de Petri contendo ágar Sabouraud com cloranfenicol em três bibliotecas de três instituições de ensino. As colônias fúngicas resultantes foram submetidas à identificação por meio da associação de aspectos macroscópicos e microscópicos, utilizando-se microcultivo. Ensaios fenotípicos complementares também foram utilizados. RESULTADOS: Das 55 amostras analisadas, foram obtidas 351 unidades formadoras de colônias (UFC), das quais 331 (94,3%) corresponderam a fungos filamentosos e 20 (5,7%) a leveduriformes. As espécies de fungos filamentosos mais frequentes foram *Penicillium* sp., *Cladosporium* sp., *Alternaria* sp., *Aspergillus* sp. e *Curvularia* sp., destacando-se maior predomínio de *Penicillium* sp. em uma biblioteca cujo ambiente não era climatizado, com 80 (22,7%) UFC. CONCLUSÃO: Os resultados deste estudo evidenciam ampla variedade de fungos com potencial patogênico e toxigênico, que podem desencadear processos alérgicos, ratificando assim a importância do estabelecimento de protocolos de higiene e de desinfecção nesse tipo de ambiente.

Palavras-chave: Fungos; Contagem de Colônia Microbiana; Bibliotecas.

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INTRODUCTION

Fungi are eukaryotic, heterotrophic, unicellular, or pluricellular organisms, widely distributed in nature, found in atmospheric air, soil, water, vegetables, debris in general, food, humans, and other animals[1,2,3,4]. The spread of these microorganisms can occur in various forms. When this propagation occurs through the air, the agents are called anemophilous fungi or allergenic fungi, whose spores are aeroallergens, responsible for respiratory manifestations, which may cause opportunistic infection in immunocompromised or high exposure hosts[1,4].

The concentration of anemophilous fungi in the environment depends on several factors; among them stand out indoor air conditioning, the intensity of human activity, and other environmental aspects, such as air current, rainfall, barometric pressure, cloudiness, temperature, relative humidity, and incidence of solar rays[2,5]. In libraries, the environment becomes conducive to the spread of microorganisms, especially anemophilous fungi, due to ventilation/air conditioning conditions, humidity, and temperature[3,6].

Studies on anemophilous mycobiota from libraries show a wide variety of fungi, including potentially pathogenic ones, whose presence represents an occupational risk and can trigger allergic manifestations in its regulars[3,4,6]. It is known that individuals confined indoors, with artificial ventilation and air conditioning, such as classrooms, libraries, theaters, cinemas, and others, may present persistent symptoms, including headaches, itching, irritation and edema of the ocular and mucous membranes, discomfort in the oropharynx and general malaise[4], compromising their quality of life.

Due to these facts, good cleaning and environmental hygiene practices are implemented in many libraries through simple measures such as using brushes, cloth (dry), or even vacuum cleaner[3]. The air quality of these indoor environments, especially when considering the characteristics of anemophilous fungi, is more affected and may present a greater number of colony-forming units (CFU) when compared to surfaces and books[6]. However, despite the existence of environmental hygiene standards, there are few studies and initiatives to care for the air quality of these places[4,7].

In this context, there is a need to consolidate information on anemophilous fungi, increase studies on microorganisms with allergenic potential, and search for environmental indicators, such as fungi. Especially in libraries, the interest arises from the implications for the conservation of books and the health condition of workers and regulars, considering that the health problems most reported by workers of this service are related to allergic mechanisms[8,9]. Therefore, this study aimed to evaluate the occurrence of anemophilous fungi in libraries of primary and higher education institutions in the city of Maceió, Alagoas State, Brazil.

MATERIALS AND METHODS

ENVIRONMENT CHARACTERIZATION AND SAMPLE COLLECTION

The samples were collected in libraries of two higher education institutions, one public and one private (spaces A and C), occupying a geographical area of 1,560 m^2 and 730 m^2, respectively, and in a library of a public school complex of primary and secondary education (space B), with an area of 850 m^2, located in Maceió. The higher education institutions had air conditioners of 12,000 BTU, while the primary and secondary education institution had only ceiling fans.

According to the researchers' observation, the three libraries presented artificial lighting, with no incidence of sunlight, and the sanitation of the floors occurred daily, using domissanitary sanitizing. In addition, with the aid of a damp cloth, dust was taken daily from wooden counters, tables, chairs, cabinets, files, book transport carts, shutters, window frames, and computers. The book covers were cleaned quarterly in space C and semiannually in spaces A and B, using cotton wetted in alcohol or ethyl acetate. The shelves were sanitized with a cloth lightly dampened with alcohol, taking care not to wet the books. Besides the cleaning described above, the dust was removed by a vacuum cleaner every two months in space C.

At the time of collection, each environment's temperature and relative humidity measurements were taken with a digital thermo-hygrometer. For the collection of anemophilous fungi, the spore sedimentation technique was used, consisting of the exposure of Petri dishes containing culture medium at the height of 1.2 m from the soil for 30 min[10]. Thus, 55 90 mm Petri dishes containing Sabouraud dextrose agar (SDA) with chloramphenicol at a concentration of 50 mg/L were strategically placed on tables, shelves, and counters, taking into account the size of the library and equidistance between two dishes of 8.5 m. Considering the same distance, 15 dishes were placed in spaces B and C and 25 dishes in space A. In space A, measuring 1,560 m^2, equal dimensions of 39.5 x 39.5 m were considered; the distance on the X-axis was 8.75 m and on the Y was 9.7 m, standardizing the equidistance of 8.5 m between the dishes, in order to respect the flow of users and employees. The same reasoning was used for spaces B and C, with an area of 850 m^2 and 730 m^2, respectively, with dimensions of 29.1 m x 29.1 m and 27 m x 27 m, for the distribution of the 15 dishes. After the exposure time, the dishes were properly labeled, packed, and transported to the Mycology Laboratory of the Centro Universitário Cesmac, where they were incubated at room temperature (28 ± 2 °C) and observed daily for seven days.

ISOLATION AND IDENTIFICATION OF FUNGI

After finding fungal growth in the dishes, quantitative analysis was performed by counting the number of CFU, differentiating them into yeast-like and filamentous.

For the isolation of fungi, purification was initially performed by transferring colony fragments to prepare a suspension in sterile distilled water, and then a streaking technique was performed in Petri dishes containing

SDA added chloramphenicol (50 mg/L). The dishes were kept at room temperature (28 ± 2 °C) to develop isolated colonies. After the growth, these colonies were transferred to test tubes containing potato dextrose agar for maintenance and later identification[11,12,13].

The identification of filamentous fungi was based on the association of macro and micromorphological characteristics of the culture. The macroscopic characteristics, such as texture, shape, and surface and reverse color of the colony, together with reproductive structures and others microscopic characteristics, such as chlamydospores, hyphae (presence or absence of septa), colors of hyphae and spores (hyaline or demáceous), were compared to the same criteria adopted by Hoog et al.[11], Lacaz et al.[12], Sidrim and Rock[13], Zaitz et al.[14], and Mezzari and Fuentefria[15]. For better identification and visualization of the structures of filamentous fungi, the microculture in a lamina was done by the technique described by Riddell[16], using Lactrimel agar to stimulate sporulation.

To identify the yeast-like fungi, macromorphological and microscopic analyses of the colonies were performed using the microculture technique in cornmeal agar. The result was associated with the germ tube test, the formation of chlamydospores in Tween 80 agar (Difco), and the assimilation and fermentation pattern of different sources of carbon and nitrogen[14].

RESULTS

At the time of the dishes' exposure, thermal records of 24.6 °C, 28.1 °C, and 21.6 °C and humidity of 68%, 69%, and 59% were observed in collections A, B, and C, respectively, totalizing 55 samples (Table 1).

Of the 55 samples analyzed, 351 CFU were obtained, of which 331 CFU (94.3%) were filamentous fungi and 20 CFU (5.7%) yeast-like fungi. The overall colony count in bibliographic collections A, B, and C resulted in 212 (60.4%), 123 (35.0%), and 16 (4.6%) CFU, respectively, according to table 2.

Library A showed a higher CFU count of both filamentous and yeast-like fungi. Regarding the identification of fungi, a higher occurrence of *Penicillium* sp. (115 CFU) was observed, followed by *Cladosporium* sp. (79 CFU), *Alternaria* sp. (20 CFU), *Aspergillus* sp. (20 CFU), and *Curvularia* sp. (18 CFU). The greatest fungal diversity was verified in library A, registering 15 genera and *Cladosporium* sp. (69 CFU) was the predominant fungus, followed by *Penicillium* sp. (32 CFU) and *Curvularia* sp. (16 CFU). In library B, where there was no air conditioning, the genus with the highest occurrence was *Penicillium* sp. (80 CFU), while in library C, the genus *Aspergillus* sp. (6 CFU) predominated. Due to the absence of reproductive structures, 13.9% (49 CFU) of fungi were not identified (Table 3).

Table 1 – Record of temperature and humidity of the environment, at the time of exposure of the dishes, and number of samples obtained from libraries A, B, and C of the city of Maceió, Alagoas State, Brazil

Library	Thermo-hygrometric parameters		Number of samples	%
	Temperature °C	RH		
A	24,6	68	25	45,4
B	28,1	59	15	27,3
C	21,6	69	15	27,3
Total			55	100,0

RH: Relative humidity.

Table 2 – Occurrence of CFU of filamentous and yeast-like fungi in libraries A, B, and C of the city of Maceió, Alagoas State, Brazil

Library	Fungi				Total	%
	Filamentous		Yeast-like			
	AF (CFU)	RF (%)	AF (CFU)	RF (%)		
A	198	56,4	14	4,0	212	60,4
B	118	33,6	5	1,4	123	35,0
C	15	4,3	1	0,3	16	4,6
Total	331	94,3	20	5,7	351	100,0

AF: Absolute frequency; RF: Relative frequency.

Table 3 – Anemophilous fungal microbiota identified in libraries A, B, and C of the city of Maceió, Alagoas State, Brazil

Genus/ Species	Libraries						Total	%
	A		B		C			
	AF (CFU)	RF (%)	AF (CFU)	RF (%)	AF (CFU)	RF (%)		
Acremonium sp.	2	0,6	–	–	2	0,6	4	1,2
Alternaria sp.	15	4,3	5	1,4	–	–	20	5,7
Aspergillus sp.	14	4,0	–	–	6	1,7	20	5,7
Bipolaris sp.	2	0,6	–	–	–	–	2	0,6
Cladosporium sp.	69	19,6	8	2,3	2	0,6	79	22,5
Curvularia sp.	16	4,6	2	0,6	–	–	18	5,2
Fonsecaea sp.	5	1,4	–	–	–	–	5	1,4
Fusarium sp.	10	2,8	–	–	–	–	10	2,8
Mycelia sterilia	–	–	1	0,3	–	–	1	0,3
Neurospora sp.	1	0,3	–	–	–	–	1	0,3
Nigrospora sp.	–	–	1	0,3	–	–	1	0,3
Penicillium sp.	32	9,1	80	22,7	3	0,8	115	32,6
Rhodotorula sp.	5	1,4	1	0,3	1	0,3	7	2,0
Scopulariopsis sp.	3	0,9	–	–	–	–	3	0,9
Trichosporon sp.	7	2,0	2	0,6	–	–	9	2,6
Ulocladium sp.	4	1,1	–	–	–	–	4	1,1
Veronaea sp.	2	0,6	1	0,3	–	–	3	0,9
Unidentified	25	7,1	22	6,2	2	0,6	49	13,9
Total	212	60,4	123	35,0	16	4,6	351	100,0

CFU: Colony-forming unit; AF: Absolute frequency; RF: Relative frequency. Conventional signal used: – Numeric data equal to zero, not resulting from rounding.

Several fungal cultures were obtained after sample collection by spontaneous sedimentation in the three libraries studied. Figure 1 shows the macroscopic and microscopic aspects of the six most prevalent fungi that exhibited more than 10 CFU in the evaluated environments, represented by *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., and *Penicillium* sp.

This study's limitations are related to the number of spaces studied and the lack of structure to perform the molecular identification of these fungi.

DISCUSSION

The results obtained in this study are similar to those reported by Menezes et al.[17], regarding fungal diversity found, since they obtained 220 CFU from 50 exhibitions in an air-conditioned library of a public higher education institution in northeastern Brazil. Air-conditioned enclosed environments tend to accumulate fungal cells dispersed in the air, as evidenced by Ribeiro and Lubisco[6] when comparing the concentration of CFU from indoor and outdoor environments of a library, where the former presented approximately twice the concentration of CFU as the second.

Libraries are spaces that provide ideal conditions for the growth of microorganisms, since they concentrate a large amount of organic matter, including paper, starch glue, leather, and fabrics[18]. Therefore, evidence indicates that, even in controlled conditions, there is contamination by fungi on surfaces and in the air[19].

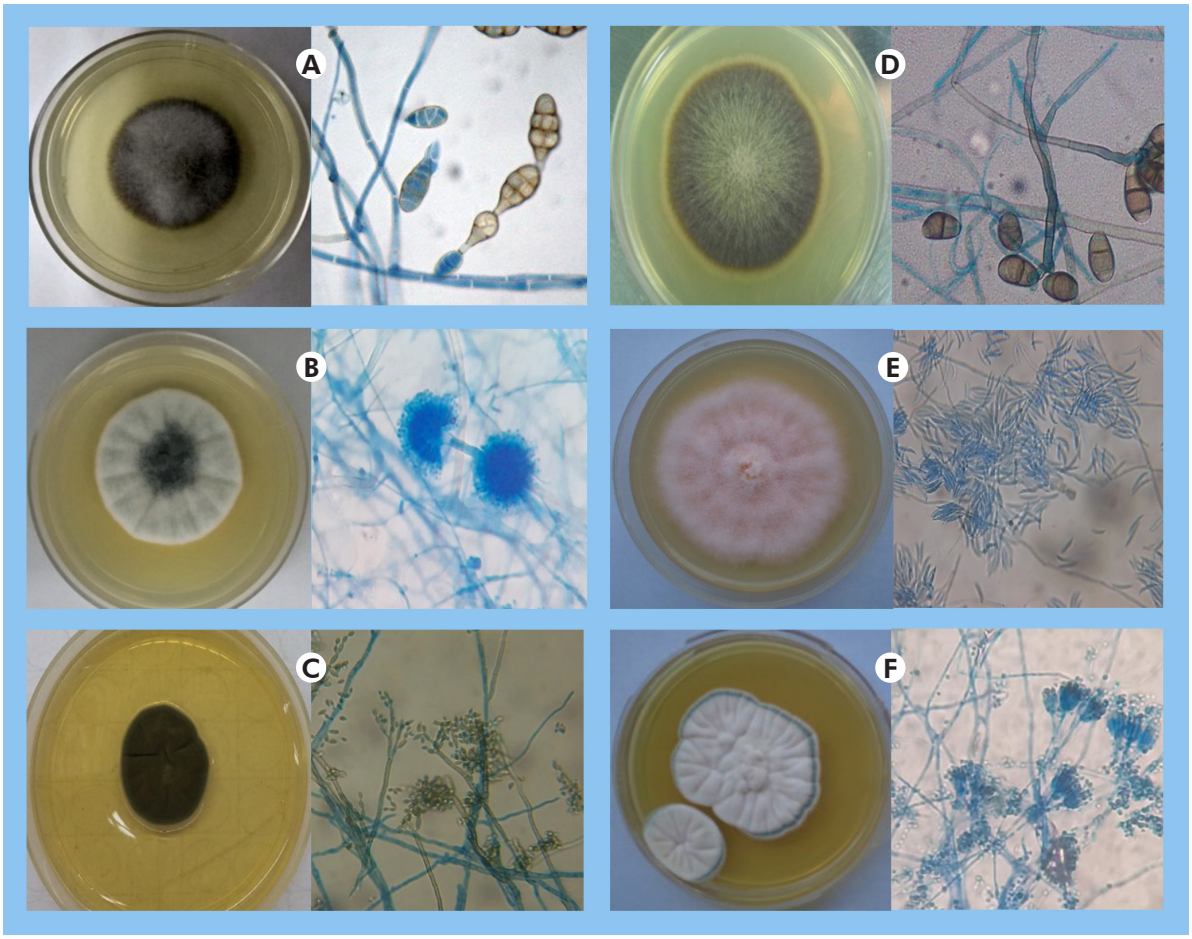
In space C, a smaller number of CFU and filamentous fungal species was observed compared to library A, which is also acclimatized. This fact may be related to the cleaning systematization at this site and the lower temperature and humidity verified at the time of collection. Library C adopted regular cleaning and hygiene practices; in addition, the environment presented better refrigeration, mainly because it had a lower flow of people on-site, an observation made during the study

period when compared to library A, which offered 88 face-to-face courses, and library C, with 13 courses and consisted of a smaller area. Thus, the higher frequency of fungi in space A may be related to greater visitation in this space by students and employees, since this library serves as a reference for other educational and research institutions in the state. Moreover, it is important to consider that the air conditioning equipment is frequently switched off at night and at weekends in this library, which causes temperature variability, mainly due to the thermal amplitudes experienced in the Northeast Region.

Second in CFU number and fungal diversity, space B did not possess air conditioning equipment and had the highest temperature during collection. Besides that, low rates of indoor air circulation can lead to an increase in the concentration of biological air pollutants[20]. Pantoja et al.[21] clarify that fungal growth occurs faster when the relative humidity of the air is above 65%, which was evidenced in environments A and B. According to Pereira and Lemos[22], temperature plays a primary function in the growth, germination, sporulation, and metabolism of fungi.

Factors such as hygiene, temperature, and luminosity are directly related to greater fungal diversity[20]. Inadequate cleaning, using methods that facilitate the aerial dispersion of microorganisms, can contribute to this fact, as occurs with brooms. It is important to consider that the fungal diversity reported in this study may be related to inefficient hygiene and air conditioning conditions, which vary in each space studied[21], with a higher prevalence of fungi in the libraries of public institutions.

In order to avoid or decrease the proliferation of fungi and other microorganisms in libraries, it is recommended to practice regular cleaning, providing the conservation of the collection, maintaining air quality, and, consequently, preserving the health of its regulars. Hygiene corresponds mainly to the removal of particulate matter (dust, pollen, human skin scales, hair, among others) from documents and surfaces of the environment and should be performed periodically, with the use of appropriate techniques[6]. The physical space of the libraries must be sufficiently airy, illuminated, and have an adequate temperature (20 to 23 °C) and humidity (55 to 65%) values for the preservation of books and documents[21]. In addition, it is essential to clean the air conditioner filter monthly, to decrease the growth of microorganisms, since the accumulation of moisture and organic material can make it a powerful dispersing source of pathogens[23,24].



A: *Alternaria* sp.; B: *Aspergillus* sp.; C: *Cladosporium* sp.; D: *Curvularia* sp.; E: *Fusarium* sp.; F: *Penicillium* sp. Blades stained with blue-cotton lactophenol.

Figure 1 – Macroscopic and microscopic aspects of fungi that showed higher prevalence in the libraries A, B and C, cultivated in Petri dishes containing potato agar dextrose at 28 °C, in the city of Maceió, Alagoas State, Brazil

Regarding the variability of fungal species, published studies corroborate the results obtained in this research because the collections carried out over the years in internal spaces of libraries showed that, among the fungi that contaminate these spaces, species of the genus *Aspergillus* and *Penicillium* predominate[8,17,20]. In the present study, the most frequent filamentous fungi species were *Penicillium* sp. and *Cladosporium* sp.; however, *Alternaria* sp., *Aspergillus* sp. and *Curvularia* sp. also presented higher frequency than other identified species.

In the last 20 years, environmental studies with air from library environments have shown that species *of Cladosporium*, *Aspergillus*, and *Penicillium* are almost ubiquitous and can produce numerous conidia that are easily dispersed by air[25]. According to Martins[26], the diversity of fungi present indoors results from the spread caused by the air currents, among other biotic factors of the external environment, and is especially related to the appearance of genera characteristic of the local microbiota of a given region.

In this sense, species belonging to the genera *Aspergillus*, *Penicillium*, and *Verticillium* present cellulolytic activity and can deteriorate materials rich in cellulose, such as wood, books, and paintings[27]. Moreover, the genera of fungi most frequently found in the air are *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Cladosporium* sp., *Curvularia* sp., *Neurospora* sp. and *Alternaria* sp.; and *Aspergillus* and *Penicillium* are considered the primary colonizers of surfaces and interiors[28].

Regarding the filamentous fungi identified in this study, most of these microorganisms are commonly associated with pathological processes. Anemophilous fungi, when inhaled, can trigger diseases ranging from respiratory allergic manifestations, such as asthma and rhinitis, to severe infections in immunocompromised individuals[20].

Among these species, fungi of the genus *Aspergillus* cause great concern, because it can cause from allergic reactions to aspergillosis, a disease characterized by a bronchopulmonary clinical picture that can spread, compromising several organs[20]. Invasive aspergillosis is related to a diversity of clinical scenarios, which commonly vary concerning manifestations, presenting a very high mortality rate[29].

Although *Penicillium* species are saprophytic, ubiquitous in the soil, survive on biodegradable organic substances, and prefer cold to moderate climates, their colonization is related to the ability to grow at a variety of temperatures, ability to survive in different ranges of water activity, and physicochemical conditions, which are found in libraries, making this environment conducive to their dispersion[30].

In addition, *Cladosporium* has been the most common fungal genus in several studies of climatized indoor environments, being considered a universal dominant fungus[31,32,33,34,35,36]. Many *Cladosporium* species are recognized as emerging pathogens, responsible for pulmonary, cutaneous infections, and respiratory system-related problems, often associated with asthmatic complaints[37,38].

Less frequently, the presence of yeast-like fungi was observed in the study environments. Although the isolation of these fungi has been reported, little attention is paid to them when compared to filamentous fungi responsible for triggering allergic processes imminently, such as *Aspergillus*. In contrast, yeast-like fungi do not immediately produce allergic or respiratory reactions, although they may be harmful to immunocompromised individuals[8]. It is essential to highlight that they may be related to superficial and systemic infections, especially in impaired immune system[39].

Trichosporon sp. presented a higher frequency among the isolated yeast-like species, with 9 CFU (2.6%). *Trichosporon* is a fungus that can be part of the normal microbiota of the human skin and gastrointestinal tract, and 17 species of this genus are clinically relevant[40,41,42] because they cause white piedra, hypersensitivity pneumonitis, and various types of invasive infections, mainly in immunocompromised individuals[41], and are therefore considered opportunistic.

Another yeast-like identified was *Rhodotorula* sp., considered commensal, found in the human airway, gastrointestinal and genital microbiota, and disseminated in the environment. It can cause opportunistic infections in immunocompromised patients, ranging from endocarditis, meningitis, ventriculitis, and peritonitis to cases of potentially fatal fungemia[43,44,45].

This study demonstrates the need to improve environmental disinfection measures to maintain the collection of the libraries and prevent diseases caused by fungi.

CONCLUSION

The results of this study show the importance of environmental disinfection routines in enclosed spaces, such as libraries, for the prevention of diseases in their participants and the conservation of the collection, since the health risk depends on the environmental control measures adopted, the cleaning frequency of the collection and environment in general, the monitoring of temperature, and relative humidity.

CONFLICTS OF INTEREST

There are no conflicts of interest in this research.

AUTHORS' CONTRIBUTION

José Rafaelly Gaia de Sousa, Delane Cristina da Silva and Maria Anilda dos Santos Araújo were responsible for the collection, processing and identification of the samples. Davi Porfirio da Silva, Rodrigo José Nunes Calumby, Lais Nicolly Ribeiro da Silva, Jayane Omena de Oliveira and Rossana Teotônio de Farias Moreira were responsible for the writing and approval of the manuscript.

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